Submission by Philip Morris U.S.A.

to

The National Toxicology Program

- Appendix 3 -

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific *N*-nitrosamine, and funded research on the metabolism of NNK

March 20, 1998

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Background

Based on experimental data available prior to 1984, the International Agency for Research on Cancer (IARC) concluded that "There is *sufficient evidence* for the carcinogenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to experimental animals," but "No data on humans were available" (IARC, 1985). In a footnote it was stated: "In the absence of adequate data on humans, it is reasonable, for practical purposes, to regard chemicals for which there is *sufficient evidence* of carcinogenicity in animals as if they presented a carcinogenic risk to humans." As far as we are aware, no regulatory agency has further evaluated the carcinogenicity of this compound to humans.

Following internal company review of all scientific literature published after the IARC assessment on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), it was concluded that:

- NNK is a carcinogen in the A/J mouse, F344 rat, and Syrian golden hamster, inducing mainly tumors of the lung, and to a lesser extent, tumors of the liver.

- Extensive evidence exists to demonstrate that human metabolism of NNK differs significantly from that observed in laboratory animals. However, relevant data have not been obtained under identical experimental conditions to allow a valid comparison of the metabolism of NNK in laboratory animal and human tissues.

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

- Current data do not support the assumption that NNK, present in tobacco products and cigarette smoke, induces lung and liver tumors in man.

This section of our submission briefly reviews published experimental data which formed the basis for our conclusions. To obtain additional scientific data on the metabolism of NNK in different animal species which would be relevant for toxicological assessment of the potential biological activity of this compound to man, Philip Morris is currently funding a research project at the Walther-Straub Institute for Pharmacology and Toxicology, University of Munich, Germany. This research will provide comparative experimental data on the *in vitro* metabolism of NNK in lung and liver of the A/J mouse, F344 rat, Syrian golden hamster, and man.

Introduction

N-Nitroso compounds represent a large diverse group of chemicals of which some, but not all, have been shown to induce a wide range of tumors in experimental animal models (Preussmann and Steward, 1984). According to recent analytical data, total human exposure to exogenous *N*-nitrosamines is estimated to be 1.10 μmol/day; the major sources are the diet (0.79 μmol/day, 80-120 μg/day; 72%), occupational exposure (0.15-0.30 μmol/day; 25%), cigarette smoking (0.02 μmol/day, 3.4 μg/day; 2%), and miscellaneous minor sources, including pharmaceutical products, cosmetics, indoor and outdoor air (0.001 μmol/day, 0.1 μg/day; 1%) (Tricker, 1997). Cigarette smoking accounts for only 2% of the estimated total exogenous exposure to *N*-nitroso compounds; however, tobacco-specific *N*-nitrosamines (TSNA), as their name implies,

PM3003531126

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

are considered to occur only in tobacco and tobacco smoke (Hecht and Hoffmann, 1989). To date seven different TSNA derived from the nitrosation of nicotine and other minor tobacco alkaloids have been identified (Amin et al., 1995). **[Figure 1]** (The following abbreviations will be used in addition to NNK: NNN, *N*-nitrosonornicotine; NAB, *N*-nitrosoanabasine; NAT, *N*-nitrosoanatabine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; iso-NNAL, 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol; and iso-NNAC, 4-(methylnitrosamino)-1-(3-pyridyl)butyric acid.)

TSNA Present in Mainstream Cigarette Smoke and Indoor Air

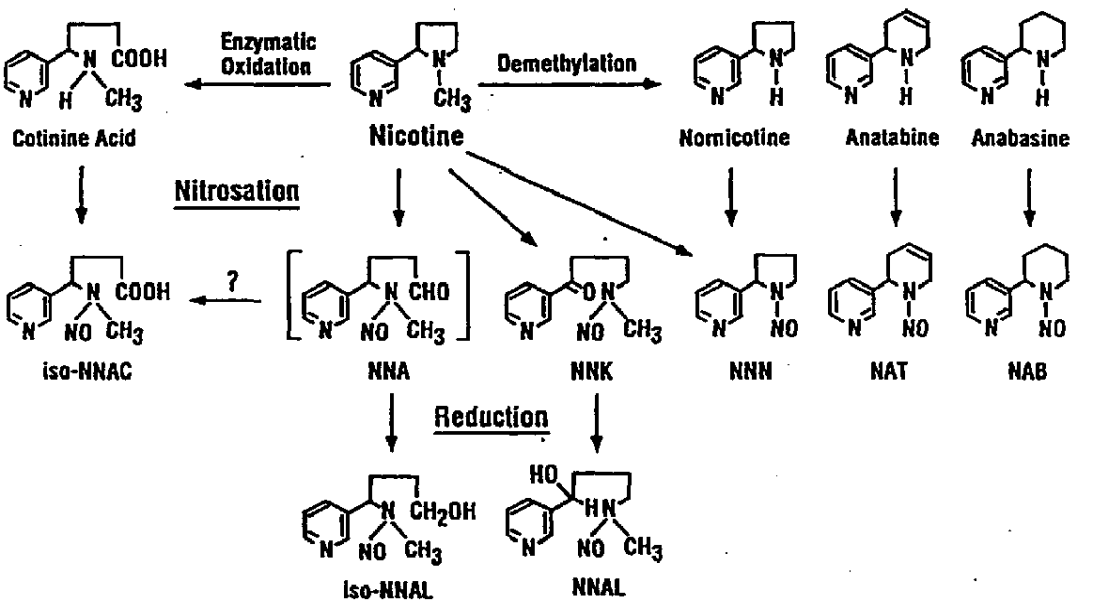
The U.S. National Academy of Sciences has previously estimated that a smoker of domestic filter cigarettes has a total *N*-nitrosamine exposure of 16.2 μg/day (6.1 μg NNN and 2.9 μg NNK), based on the assumption that the average smoker consumes 20 cigarettes/day (Assembly of Life Sciences, 1981). This exposure estimate was based on unpublished analytical data provided by Hecht and Hoffmann of the American Health Foundation, Valhalla, NY. More recent data for filter cigarettes **[Table 1]** yields a lower exposure estimate of 3.4 μg/day (1.5 μg NNN and 1.0 μg NNK) (Tricker, 1997).

Since TSNA are also transferred to sidestream cigarette smoke (Adams et al., 1987), and presumably exhaled by smokers, trace levels occur in environmental tobacco smoke (ETS) present in indoor air (Brunnemann et al., 1992; Klus et al., 1992; Tricker et al., 1994). Extensive smoking under poor ventilation conditions results in mean ETS concentrations of 2.8±1.6 (range n.d.-6.0) ng/m^3 NNN and 4.9±9.6 (range n.d.-13.5) ng/m^3 NNK (Klus et al., 1992). Similar levels

PM3003531127

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Figure 1. **Proposed formation of tobacco-specific *N*-nitrosamines.**



PM3003531128

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 1. Estimates of exposure to TSNA in smokers.

	Exposure estimate (μg/day) For a smoker of 20 filter cigarettes		
TSNA	1981[1]	1991[2]	1994[3]
NNK	3.0	1.0	1.6
NNN	6.2	1.5	1.0
NAB	--	--	0.2
NAT	7.4	--	1.8
NAB/NAT	--	1.5	--

1. Academy of Life Sciences (1981).

2. Tricker et al. (1991).

3. Hoffmann et al. (1994).

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

occur in the home; 0.8±1.2 (range n.d.-3.3) ng/m^3 NNN and 4.0±4.6 (range n.d.-14.3) ng/m^3 NNK (Tricker et al., 1994), and other venues (Brunnemann et al., 1992).

Putative Metabolism of NNK in Laboratory Animals

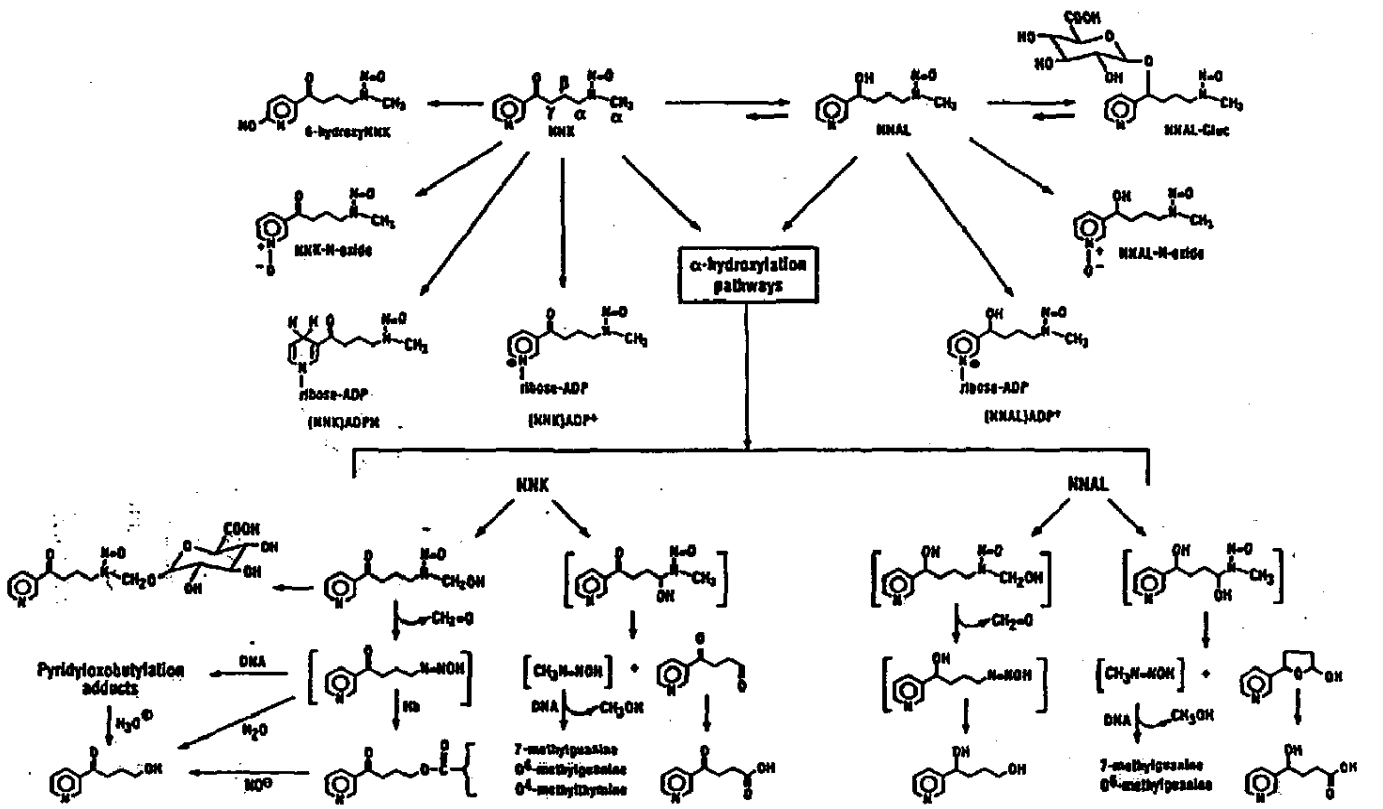
The major reported pathways of NNK metabolism in experimental animals involve carbonyl reduction, α-hydroxylation of the methylene and methyl groups adjacent to the *N*-nitroso group, and pyridine-N-oxidation **[Figure 2]**.

Carbonyl reduction of NNK to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (**NNAL**) is probably a detoxification pathway of NNK metabolism since it provides the functional hydroxy moiety necessary for glucuronidation to [4-(methylnitrosamino)-1-(3-pyridyl)but-1-yl]-β-O-D-glucosiduronic acid (**NNAL-Gluc**) (Maser et al., 1996; Kim and Wells, 1996). α-Hydroxylation of the NNK methyl carbon results in the formation of an unstable intermediate which spontaneously decomposes to yield formaldehyde and 4-(3-pyridyl)-4-oxobutanediazohydroxide, a potential pyridyloxobutylating agent, which can react with water to yield 4-hydroxy-1-(3-pyridyl)-1-butanone (**keto alcohol**). α-Hydroxylation of the methylene group in NNK produces 4-hydroxy-4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone which spontaneously decomposes to methane diazohydroxide, a potential methylating species, and stable 4-oxo-1-(3-pyridyl)-1-butanone (**keto aldehyde**), which is further oxidized to 4-oxo-4-(3-pyridyl)butyric acid (**keto acid**). Glucuronidation of 4-((hydroxymethyl)nitrosamino)-1-(3-pyridyl)-1-butanone, the initial

PM3003531130

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Figure 2. **Putative metabolism of NNK in laboratory animals (Hecht, 1996).**



PM3003531131

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

intermediate formed in the "keto alcohol" pathway, may be a detoxification route (Murphy et al., 1995).

α-Hydroxylation of the methylene and methyl groups adjacent to the *N*-nitroso group in NNAL produces unstable metabolic intermediates before ultimately forming stable 4-hydroxy-4-(3-pyridyl)butric acid (**hydroxy acid**) and 4-hydroxy-1-(3-pyridyl)-1-butanol (**diol**). α-Hydroxylation of the methyl group and ultimate formation of the diol is a putative detoxification pathway since this pathway has not been reported to result in adduct formation, while α-hydroxylation of the methylene group ("hydroxy acid" pathway) can potentially result in methylation of cellular macromolecules. NNAL is a poor substrate for α-hydroxylation compared to NNK (Hecht and Trushin, 1988; Belinsky et al. 1989; Staretz et al. 1997a), and consequently exhibits less biological activity than NNK (Liu et al., 1990; Castonguay et al., 1983a; Hoffmann et al., 1993).

Pyridyl-N-oxidation of both NNK and NNAL to yield 4-(methylnitrosamino)-1-(3-pyridyl-N-oxide)-1-butanone (**NNK-N-oxide**) and 4-(methylnitrosamino)-1-(3-pyridyl-N-oxide)-1-butanol (**NNAL-N-oxide**), respectively, are considered to be detoxification pathways of NNK metabolism (Liu et al., 1990; Castonguay et al., 1983a; Hecht, 1994; Staretz et al., 1997a).

The determination of hydroxy acid, keto acid and keto alcohol as stable end products of NNK metabolism by α-hydroxylation pathways represents the metabolic activation of the host to produce reactive intermediates with the potential to form adducts with DNA or other cellular macromolecules.

PM3003531132

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Urinary NNK Metabolite Excretion in Laboratory Animals and Man

Urinary excretion profiles provide evidence of species-dependent differences in metabolic activation of NNK by α-hydroxylation and detoxification **[Table 2]**.

Urinary NNK metabolite profiles in the A/J mouse and F344 rat (Morse et al., 1990) are highly dose dependent **[Table 3]**. Similar studies have not been performed in other animal species. The data in **Table 3** indicate that α-hydroxylation pathways account for about 50% of NNK metabolism in the A/J mouse and F344 rat, regardless of the administered dose. At the lowest administered dose of NNK (1 μg/kg body weight), excretion of NNAL and NNAL-Gluc does not occur in either species, although NNAL is apparently formed and further metabolized by pyridyl-N-oxidation. NNK-N-oxide excretion increases with decreasing administration of NNK.

Human biomonitoring studies report the presence of NNAL, NNAL-Gluc and NNAL-N-oxide in 24-h urine of smokers maintaining constant smoking habits (Hecht et al. 1995; Carmella et al., 1997). NNK-N-oxide is not a urinary metabolite of NNK in smokers (Carmella et al., 1997). Other metabolites of NNK detected in experimental animal excretion studies, such as stable end products of α-hydroxylation , are not specific to NNK metabolism since they are also formed during metabolism of NNN and nicotine. Combined urinary excretion of NNAL, NNAL-Gluc and NNAL-N-oxide in urine of smokers (Hecht et al., 1995; Carmella et al., 1997), when calculated in molar equivalents of NNK, balances well with predicted total NNK exposure **[Table 4]**. These data

PM3003531133

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 2. Urinary excretion of NNK metabolites in different species.

	% urinary excretion of NNK metabolites			
Metabolite	A/J mice[1] 0.1 mg/kg i.p. 48-h excretion	F344 rat[2] 0.1 mg/kg i.p. 24-h excretion	F344 rat[1] 0.1 mg/kg i.p. 48-h excretion	Patas monkey[3] 0.1 mg/kg i.v. 24-h excretion
Hydroxy acid*	34	16.0	28	41.9-42.9
Keto acid*	19	37.8	21	24.6-26.6
Keto alcohol	--	<0.06	--	--
NNAL	1	3.3	7	n.d.-2.0
NNAL-Gluc	3	0.6	2	19.1-19.9
NNK-N-oxide	8	11.7	7	13.6-15.7
NNAL-N-oxide	14	11.4	16	7.7-15.7
NNK	--	0.8	--	n.d.-0.1
6-hydroxy-NNK	--	1.0	--	--
Total α-hydroxylation*	53	54	49	58.1

1. Morse et al. (1990).
2. Murphy et al. (1995).
3. Hecht et al. (1993a).

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 3. Dose response for excretion of NNK metabolites.[1]

Metabolite	% 24-h excretion of NNK metabolites at different dose levels (mg/kg i.p.)					
	103.5	10.35	1.035	0.103	0.010	0.001
A/J mouse:						
Hydroxy acid*	18	32	37	34	27	35
Keto acid*	11	16	26	19	27	23
NNAL	29	11	2	1	--	--
NNAL-Gluc	22	8	4	3	--	--
NNK-N-oxide	--	--	5	8	10	7
NNAL-N-oxide	11	13	8	14	7	6
Total α-hydroxylation*	29	48	63	53	54	58
F344 rat:						
Hydroxy acid*	26	24	20	28	16	14
Keto acid*	24	39	45	21	38	40
NNAL	25	12	6	7	4	--
NNAL-Gluc	8	3	2	2	2	--
NNK-N-oxide	3	3	6	7	14	14
NNAL-N-oxide	6	6	8	16	13	12
Total α-hydroxylation*	50	63	66	49	54	54

1. Morse et al. (1990).

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 4. Biomonitoring of NNK metabolites in human urine.

Smoker	Cigarettes/day	Total excretion of NNK Metabolites (nmol/day)[1]	Theoretical NNK exposure and excretion Exposure (μg/day)[2]	Excretion (μg/day)
1	18.2±2.2	7.32	0.91-1.46	1.52
2	16.7±1.1	6.24	0.85-1.34	1.29
3	16.8±1.1	4.63	0.84-1.34	0.96
4	15.0±1.1	4.68	0.75-1.20	0.97
5	15.8±0.4	4.75	0.79-1.26	0.98
6	9.5±1.2	Data incomplete	--	--
7	14.2±1.1	2.41	0.71-1.14	0.50
8	13.6±0.7	Data incomplete	--	--
9	19.1±1.7	4.30	0.95-1.53	0.89
10	8.0±1.4	3.74	0.40-0.64	0.77
11	15.9±1.0	Data incomplete	--	--
Mean			0.78-1.24	0.99
S.D.				0.31

1. Total excretion of NNAL, NNAL-Gluc and NNAL-N-oxide. To convert to μg NNK multiply by 207.

2. Estimated exposure range based on mainstream cigarette smoke NNK concentrations (Tricker et al., 1991; Hoffman et al., 1994).

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

provide evidence that NNK metabolism and excretion in man differs significantly from that observed in laboratory animals **[Table 2]**.

Total NNAL plus NNAL-Gluc excretion in 5 nonsmokers measured on 2 occasions is reported to be 8.4±11.2 ng/day, and increases after experimental exposure to sidestream smoke used as a surrogate for ETS (Hecht et al., 1993b). Another study reported 17 of 29 nonsmokers to have a mean excretion of 8.8±9.4 (range 0.8-31) ng/day total NNAL plus NNAL-Gluc compared to 0.68±0.41 (range 0.08-1.68) μg/day in smokers (Meger et al., 1998). Twelve of 29 nonsmokers had no detectable levels of NNAL plus NNAL-Gluc in urine.

Metabolism of NNK by Laboratory Animal and Human Microsomes

Microsomes from lung and liver have been extensively used to investigate metabolism of NNK under various experimental conditions **[Appendix 1]**. Despite differences in the individual study protocols (NNK substrate concentration and time of incubation), rodent lung and liver microsomes metabolize NNK to yield significant levels of α-hydroxylation products. Contrary to this, lung and liver microsomes of human origin primarily metabolize NNK by keto reduction to NNAL in the absence of significant α-hydroxylation. Only limited kinetic data are available to document interspecies differences in NNK metabolism by lung and liver microsomes.

PM3003531137

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

The available kinetic parameters for NNK metabolism by lung microsomes **[Table 5]** provide further indication that laboratory animals (A/J mouse and patas monkey) primarily metabolize NNK by α-hydroxylation with no significant formation of NNAL. Human lung microsomes primarily metabolize NNK to NNAL, with no significant formation of α-hydroxylation products. The biological relevance of data for metabolism of NNK by human lung microsomes is partially comprised by the high experimental substrate range compared to actual human exposure to NNK (19-135 ng/filter cigarette; 70-650 pmol [Tricker et al., 1991]). No data are available for microsomes isolated from rat lung.

Metabolism of NNK by hepatic microsomes is less well documented **[Table 6]**. Liver microsomes from the patas monkey primarily metabolize NNK via α-hydroxylation with no significant formation of NNAL at low NNK substrate concentrations, while at high substrate concentrations metabolism to NNAL would be predicted to occur. Human liver microsomes primarily metabolize NNK to NNAL, and to a lesser extent NNAL-N-oxide, at low substrate concentrations, with no significant formation of α-hydroxylation products. The formation of NNAL-N-oxide at low substrate concentrations supports the presence of this metabolite in human urine (Carmella et al., 1997). Only kinetic parameters for NNK α-hydroxylation pathways have been reported for A/J mouse and F344 rat liver microsomes.

In conclusion, kinetic parameters of NNK metabolism in lung and liver microsomes provide strong evidence that significant differences occur in metabolism between laboratory animals and man. At low levels of exposure to NNK, human metabolism is characterized by reduction of

PM3003531138

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 5. Metabolism of NNK by lung microsomes.

Kinetic parameters: *Km* (μM), *Vmax* (pmol/min/mg protein)

Metabolite	Mouse[1]		Rat		Patas monkey[2]		Human[3]	
	Km	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*
Keto alcohol	5.6	56			4.9	19.1	--	--
Keto aldehyde	--	--			10.3	5.3	653	4.6
Keto acid	9.2	4.2	No published		--	--	--	--
Hydroxy acid	--	--	data		--	--	526	2.9
NNAL	2541	1322			902	479	573	335
NNAL-N-oxide	4.7	54			--	--	--	--
NNK-N-oxide	--	--			5.4	19.1	531	7.7
Substrate range	1-100 μM NNK				1-20 μM NNK		7-200 μM NNK	

1. Smith et al. (1990).
2. Smith et al. (1997).
3. Smith et al. (1992).

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 6. Metabolism of NNK by liver microsomes.

Kinetic parameters: *Km* (μM), *Vmax* (pmol/min/mg protein)

Metabolite	Mouse[1]		Rat[2]		Patas monkey[3]		Human[4]	
	Km	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*
Keto alcohol	73.8	239	211	156	474	37.7	1200	500
Keto aldehyde	19.1	173	234	153	8.2	37.4	367	60
NNAL	--	--	--	--	474	3470	56	282
							1600	3300
NNAL-N-oxide	--	--	--	--	--	--	53	19
							4500	560
NNK-N-oxide	--	--	--	--	--	--	--	--
Substrate range	1-100 μM NNK		1-200 μM NNK		1-50 μM NNK		5-2000 μM NNK	

1. Peterson et al. (1991).
2. Guo et al. (1992).
3. Smith et al. (1997).
4. Patten et al. (1996).

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

NNK to NNAL, in the absence of significant α-hydroxylation, while laboratory animals metabolize NNK to yield significant levels of α-hydroxylation products.

Metabolism of NNK by Laboratory Animal and Human Tissues

Similar profiles are observed for metabolism of NNK by microsomes and tissue samples **[Appendix 1]**. The data confirm that significant differences occur between laboratory animal and human metabolism of NNK. The major pathways of NNK metabolism in rodent tissues yield significant levels of α-hydroxylation products while human tissues primarily reduce NNK to NNAL.

DNA and Hemoglobin Adduct Formation by NNK

Metabolism of NNK by α-hydroxylation is assumed to be a critical event resulting in DNA-reactive intermediates; however, the exact role of methylating and pyridyloxobutylating species in different animal species and organs remains unclear. Metabolism of NNK to the keto acid via a methylating intermediate is thought to be a critical determinant of NNK-induced tumorigenesis in the A/J mouse lung (Peterson and Hecht, 1991; Belinsky et al., 1992) and hamster liver (Liu et al., 1992). Although NNK-induced methylation to yield O^6-methylguanine in Clara cells appears to be a suitable indicator of the carcinogenic potency of NNK in the rat lung (Belinsky et al., 1990), pyridyloxobutylation is thought to be the critical event in tumor induction in rat lung (Staretz et al., 1997b) and liver (Liu et al., 1992). Both NNK and NNN can be metabolized to a

PM3003531141

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

pyridyoxobutylating species in the rat lung (Hecht et al., 1988) to yield a N^2-(pyridyloxobutyl)deoxyguanosine (HPB) adduct with DNA (Spratt et al., 1989). Linear dose-response relationships are not observed for either NNK-induced methylation (Belinsky et al., 1990; Murphy et al., 1990a) or NNK-induced pyridyloxobutylation in the rat lung (Murphy et al., 1990a). An HPB-releasing adduct with hemoglobin has been proposed as a surrogate marker for DNA adduct formation by both NNK and NNN (Carmella and Hecht, 1987; Peterson et al., 1990). However, the exact mechanism of adduct formation still remains to be determined (Murphy and Coletta, 1993).

Only two studies have investigated the presence of HPB-releasing DNA adducts in human lung (Foiles et al., 1991; Blömeke et al., 1996). Mean levels of 11±16 and 9.0±2.3 fmol HPB/mg DNA have been reported in smokers and nonsmokers, respectively (Foiles et al., 1991). Since the response for the analytical reagent blank was equivalent to 38±16 fmol HPB and 1-2 mg DNA were used for analysis, the reported levels are well below the blank response and could easily be due to an analytical artifact. No HPB-releasing DNA adducts were detected in 16 lung tissue samples from current smokers and 16 from nonsmokers (Blömeke et al., 1996). The levels of 7-methylguanine in 80 lung tissue samples could not be explained by differences in tobacco exposure (measured by serum cotinine), gender, age, or ethnicity (Blömeke et al., 1996). These data suggest that exposure to NNK via smoking does not result in HPB adduction to lung DNA or increase the background level of lung DNA methylation.

Determination of hemoglobin adducts as a possible surrogate for tissue DNA adducts shows less than a 3-fold difference in the mean level of HPB-releasing hemoglobin adducts in

PM3003531142

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

smokers (163 fmol HPB/g hemoglobin) compared to nonsmokers (68 fmol HPB/g hemoglobin) (Hecht, 1994). A smaller difference was observed in another study (54.7±8.9 vs. 26.7±4.1 fmol/g hemoglobin) and self-reported exposure of nonsmokers to environmental tobacco smoke did not increase the background level of HPB-releasing hemoglobin adducts (Richter et al., 1995). Both studies indicated elevated hemoglobin adduct levels were only apparent in about 10% of smokers compared to all subjects.

Concluding Remarks

The above studies provide intensive evidence that human metabolism of NNK differs significantly from that observed in laboratory animals. These differences are evident from *in vitro* studies of NNK metabolism in microsomes and tissue samples, excretion profiles of NNK metabolites, and absence of a clear differentiation in NNK/NNN-derived DNA and hemoglobin adducts in smokers and nonsmokers. These data provide little support for the assumption that NNK in tobacco products and cigarette smoke induces similar biological effects in the lung and liver as reported in laboratory animals.

PM3003531143

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Research Funded by Philip Morris on the Metabolism of NNK

A research project has been funded at the Walther-Straub Institute for Pharmacology and Toxicology, University of Munich, Germany. The research has two major objectives:

1. To define the major routes of *in vitro* NNK and NNAL metabolism under identical experimental conditions in lung and liver of the A/J mouse, F344 rat, Syrian golden hamster, and man. The three animal species chosen represent those most often used for chronic bioassays of NNK.

2. To determine pharmacokinetic constants (*Km* and *Vmax*) for each pathway of NNK and NNAL metabolism in both organs of all four species.

Experimental Design

Lung and liver are removed from laboratory animals killed by decapitation. Human peripheral lung and liver tissue are collected from excess material removed at surgery from patients undergoing routine surgical procedures. Human tissues are rejected from subjects having current treatment with immuno-suppressants or other drugs known to induce or suppress metabolism, or a history of alcohol abuse. Undamaged macroscopically normal tissue is received in the laboratory stored in ice-cold Hanks medium within 30 min of removal.

PM3003531144

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Metabolic studies are performed using precision cut liver slices and lung tissue in dynamic culture (Vickers, 1994). Compared to using microsomes or isolated cells (extensively used in previous studies as summarized in **Appendix I**), tissue samples maintain structural heterogeneity with intact phase I and phase II metabolism, and cell interaction and communication are preserved to a certain extent, thus resembling the situation in the intact organ.

Radiolabeled [5-^{3}H]NNK or [5-^{3}H]NNAL (sp. act. 25-30 Ci/mmol) are incubated over a substrate concentration range of 0.01-100.0 μM with precision cut liver slices and lung tissue for 6 h in Krebs-Henseleit buffer (pH 7.4) under standard laboratory conditions for dynamic culture (Vickers, 1994). The substrate concentration range was selected to include the lowest possible concentration to approach the physiologically relevant concentration in man, and substrate concentrations predicted to occur in animal bioassays. Each incubation is performed in triplicate using lung and liver samples from at least 5 different laboratory animals. Metabolite profiles are determined by reversed-phase HPLC with radioflow detection (Richter and Tricker, 1994). Pharmacokinetic parameters (*Km* and *Vmax*) are calculated from Lineweaver-Burk plots with reaction velocities showing linear response to time. Pharmacokinetic parameters will be determined for lung and liver from different human donors.

Current Results

Preliminary results from this research have already been presented at two scientific meetings:

PM3003531145

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

1. AACR Special Conference in Cancer Research "DNA methylation, imprinting, and the epigenetics of cancer," Las Croabas, Puerto Rico, December 12-16, 1997.

2. 37th Annual Meeting of the Society of Toxicology, Seattle, March 1-5, 1998.

Further results will be presented at the 89th Annual Meeting of the American Association for Cancer Research, New Orleans, March 28-April 1, 1998.

The data currently available from this research project **[Table 7, Table 8]** demonstrate that NNK metabolism in lung of the A/J mouse and F344 rat results in significant levels of α-hydroxylation products at high tissue substrate concentrations predicted to occur in animal bioassay protocols. The experimental conditions and data support the reported biological activity of NNK in the A/J mouse and F344 rat lung; metabolism of NNK at high substrate concentrations yields the keto acid via a methylation pathway in the A/J mouse lung, while formation of the keto alcohol via the pyridyloxobutylation pathway occurs in the F344 rat lung. These two pathways are considered critical for lung tumorigenesis in A/J mouse lung (Peterson and Hecht, 1991; Belinsky et al., 1992) and F344 rat lung (Staretz et al., 1997b), respectively. Metabolism of NNK by human lung results primarily in the formation of NNAL in the absence of formation of significant levels of α-hydroxylation products **[Figure 3]**. The kinetic data **[Table 7]** provide support for pyridyl-N-oxidation of NNAL, but not NNK, and are consistent with the reported occurrence of NNAL-N-oxide but not NNK-N-oxide in human urine (Carmella et al., 1997). The kinetic data do not support significant formation of keto alcohol via the pyridyloxobutylation pathway at low NNK substrate

PM3003531146

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 7. Metabolism of NNK in lung tissue.

Kinetic parameters: *Km* (μM), *Vmax* (pmol/min/mg protein)

Metabolite	A/J Mouse		SG Hamster		F344 Rat		Human	
	Km	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*
0.01-1.0 μM NNK								
NNAL	1.7	168	No	No	3.6	309	1.0	311
Keto acid	0.3	45	current	current	5.2	182	0.4	11
Keto alcohol	0.6	141	data	data	2.7	177	1.2	26
Hydroxy alcohol	1.0	56			n.d.	--	0.7	25
NNK-N-oxide	0.7	184			6.8	498	n.d.	n.d.
NNAL-N-oxide	n.d.	n.d.			n.d.	--	0.6	19
0.01-100μM NNK								
NNAL	39.0	4309			--	--	239	65640
Keto acid	10.1	317			317.0	15620	--	--
Keto alcohol	7.5	556			90.0	8398	--	--
Hydroxy alcohol	160.0	3405			2.4	107	--	--
NNK-N-oxide	25.1	25.1			68.0	7995	41240	413500
NNAL-N-oxide	n.d.	n.d.			0.9	21	--	--

Substrate range: 0.01-100.0μM NNK

n.d., no detectable formation; --, Michaelis-Menton kinetics could not be fitted.

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Table 8. Metabolism of NNK in liver tissue.

Kinetic parameters: *Km* (μM), *Vmax* (pmol/min/mg protein)

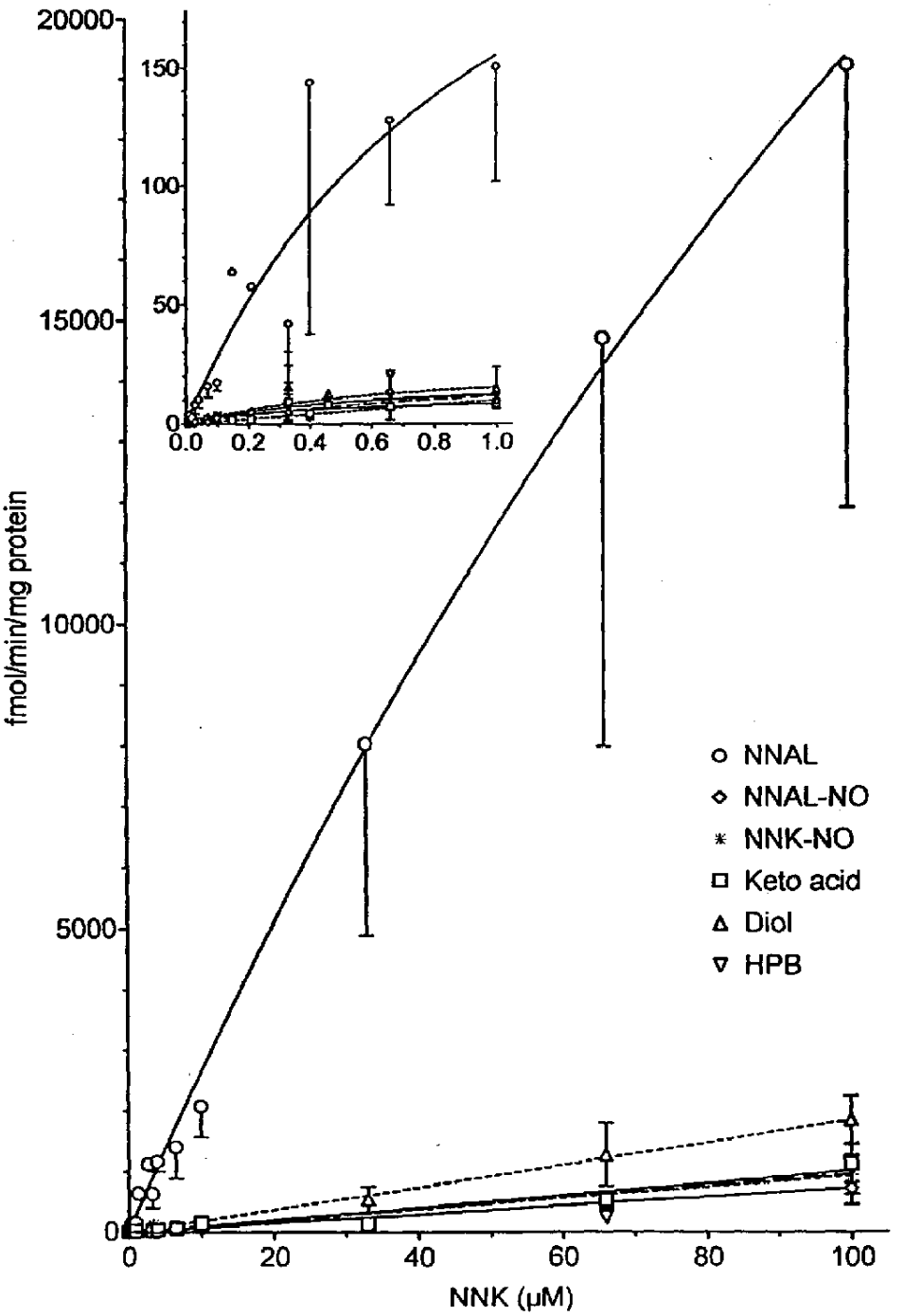
Metabolite	A/J Mouse		SG Hamster		F344 Rat		Human	
	Km	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*	*Km*	*Vmax*
0.01-1.0 μM NNK								
NNAL	--	--	No current data	No current data	26.0	4640	0.6	254
Keto acid	--	--			0.9	18	0.2	9.6
Keto alcohol	--	--			1.9	62	n.d.	n.d.
Hydroxy alcohol	--	--			n.d.	n.d.	--	--
0.01-100μM NNK								
NNAL	174.0	4623			96.0	19220	43.6	6805
Keto acid	677.0	5818			154.0	1159	690.0	8036
Keto alcohol	141.0	2609			260.0	8357	12760	144500
Hydroxy alcohol	199.0	3153			16.2	253	n.d.	n.d.

Substrate range: 0.01-100.0μM NNK

n.d., no detectable formation; --, Michaelis-Menton kinetics could not be fitted.

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Figure 3. **Metabolism of NNK by 7 different human lungs.**



PM3003531149

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

concentrations in human lung. These data suggest that HPB-releasing adducts derived from NNK are unlikely to be formed in the human lung and support data for their absence in human lung tissue (Blömeke et al., 1996).

Michaelis-Menton kinetics could not be fitted to NNK metabolism at low substrate concentrations in the A/J mouse liver. At high substrate concentrations (1.0-100 μM), significant metabolism of NNK by α-hydroxylation pathways occurs in addition to NNAL formation. NNK metabolism in the liver of the F344 rat suggested that hydroxy acid formation is the most favorable pathway for NNK metabolism; however, under conditions used in rat bioassay protocols, significant α-hydroxylation of NNK still occurs. At low physiological NNK substrate concentrations in the human liver, NNK metabolism is predicted to result primarily in the formation of NNAL.

In summary, these results obtained under identical experimental conditions provide further evidence that NNK metabolism in human lung and liver primarily yields NNAL. In contrast to this, NNK metabolism in lung and liver of the A/J mouse and F344 rat results in significant α-hydroxylation to DNA-reactive intermediates thought to be involved in NNK-induced tumorigenesis in these two organs.

The research program is predicted to be completed in April 1998.

PM3003531150

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

References

Amin, S., Desai, D., Hecht, S.S., Hoffmann, D., Synthesis of tobacco-specific N-nitrosamines and their metabolites and results of related bioassays, *Crit. Rev. Toxicology* 28: 139-147 (1996).

Ardries, C.M., Smith, T.J., Kim, S., Yang, C.S., Induction of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) activation in rat lung mincosomes by chronic ethanol consumption and repeated running exercise, *Cancer Lett.* 103: 209-218 (1996).

Assembly of Life Sciences, *The Health Effects of Nitrate, Nitrite and N-Nitroso Compounds*, National Academy Press, Washington, D.C., Chapter 7 (1981).

Belinsky, S.A., White, C.M., Trushin, N., Hecht, S.S., Cell specificity for the pulmonary metabolism of tobacco-specific nitrosamines in the Fischer rat, *Carcinogenesis* 10: 2269-2274 (1989).

Belinsky, S.A, Foley, J.F., White, C.M., Anderson, M.W., Maronpot, R.R., Dose-response between O^6-methylguanine formation in Clara cells and induction of pulmonary neoplasia in the rat by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *Cancer Research* 50: 3772-2780 (1990).

Belinsky, S.A., Devereux, T.R., Foley, J.F., Maronpot, R.R., Anderson, M.W., Role of the alveolar type II cell in the development and progression of pulmonary tumors induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the A/J mouse, *Cancer Research* 52: 3164-3173 (1992).

Blömeke, B., Greenblatt, M.J., Doan, V.D., Bowman, E.D., Murphy, S.E., Chen, C.C., Kato, S., Shields, P.G., Distribution of 7-alkyl-2'-deoxyguanosine adduct levels in human lung, *Carcinogenesis* 17: 741-748 (1996).

Bouchard, L., Castonguay, A., Inhibitory effects of nonsteroidal anti-inflammatory drugs (NSAIDs) on the metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in mouse lung explants, *Drug Met. Dis.* 21: 293-298 (1993).

Brittebo, E.B., Castonguay, A., Furuya, K., Hecht, S.S., Metabolism of tobacco-specific nitrosamines by cultured rat nasal mucosa, *Cancer Res.* 43: 4343-4348 (1983).

Brunnemann, K.D., Cox, J.E., Hoffmann, D., Analysis of tobacco-specific N-nitrosamines in indoor air, *Carcinogenesis* 13: 2415-2418 (1992).

Carmella, S.G., Hecht, S.S., Formation of hemoglobin adducts upon treatment of F344 rats with the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine, *Cancer Res.* 47: 2626-2630 (1987).

PM3003531151

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Carmella, S.G., Borukhova, A., Akerkar, S.A., Hecht, S.S., Analysis of human urine for pyridine-N-oxide metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific lung carcinogen, *Cancer Epidemiology Biomarkers Prev.* 6: 113-120 (1997)

Castonguay, A., Rossignol, G., Modulation of the activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by hamster liver microsomes to protein alkylating species, *Toxic. in Vitro* 6: 397-404 (1992).

Castonguay, A., Lin, D., Stoner, G.D., Radok, P., Furyua, K., Hecht, S.S., Schut, H.A.J., Klaunig, J.E., Comparative carcinogenicity in A/J mice and metabolism by cultured mouse peripheral lung of N'-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and their analogues, *Cancer Res.* 43: 1223-1229 (1983a).

Castonguay, A., Stoner, G.D., Schut, H.A.J., Hecht, S.S., Metabolism of tobacco-specific N-nitrosamines by cultured human tissues, *Proc. National Academy of Sciences USA* 89: 6694-6697 (1983b).

Charest, M. Rossignol, G., Castonguay, A., In vitro and in vivo modulation of the bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in hamster lung tissue, *Chem.-Biol. Interactions* 71: 265-278 (1989).

Desai, D., Numes, M., Chang, L., Lin, J.M., Jiao, D., Amin, S., Ipomeanol analogs as chemopreventive agents: Effect on in vitro metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), *Cancer Lett.* 97: 156-162 (1995).

El-Bayoumy, K., Prokopczyk, B., Peterson, L.A., Desai, D., Amin, S., Reddy, B.S., Hoffmann, D., Wynder, E., Effects of dietary fat content on the metabolism of NNK and DNA methylation induced by NNK, *Nutr. Cancer* 26: 1-10 (1996).

Foiles, P.G., Akerkar, S.A., Carmella, S.G., Kagan, M., Stoner, G.D., Resau, J.H., Hecht, S.S., Mass spectrometric analysis of tobacco-specific nitrosamine-DNA adducts in smokers and nonsmokers, *Chem. Res. Toxicol.* 4: 364-368 (1991).

Guo, Z., Smith, T.J., Thomas, P.E., Yang, C.S., Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by inducible and constitutive cytochrome P450 enzymes in rats, *Arch. Biochem. Biophys.* 298: 279-286 (1992).

Hamilton, S.M., Teel, R.W., Effects of isothiocyanates on the metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo[a]pyrene by hamster and rat liver microsomes, *Anticancer Res.* 14: 1089-1094 (1994).

Hecht, S.S., Metabolic activation and detoxification of tobacco-specific nitrosamines -- A model for cancer prevention strategies, *Drug. Met. Rev.* 26: 373-390 (1994).

PM3003531152

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Hecht, S.S., Recent studies on mechanisms of bioactivation and detoxification of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific lung carcinogen, *Crit. Rev. Toxicol.* 26: 163-181 (1996).

Hecht, S.S, Hoffmann, D., The relevance of tobacco-specific nitrosamines to human cancer, *Cancer Surv.* 8: 273-294 (1989).

Hecht, S.S., Trushin, N., DNA and hemoglobin alkylation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its major metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol in F344 rats, *Carcinogenesis* 9: 1665-1668 (1988).

Hecht, S.S., Spratt, T.E., Trushin, N., Evidence for 4-(3-pyridyl)-4-oxobutylation of DNA in F344 rats treated with the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine, *Carcinogenesis* 9: 161-165 (1988).

Hecht, S.S., Trushin, N., Reid-Quin, C.A., Burak, E.S., Jones, A.B., Southers, J.L., Gombar, C.T., Carmella, S.G., Anderson, L.M., Rice, J.M., Metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in the patas monkey: Pharmacokinetics and characterization of glucuronide metabolites, *Carcinogenesis* 14: 229-236 (1993a).

Hecht, S.S., Carmella, S.G., Murphy, S.E., Akerkar, S., Brunnemann, K.D., Hoffmann, D., A tobacco-specific lung carcinogen in the urine of men exposed to cigarette smoke, *New England Journal of Medicine* 329: 1543-1546 (1993b).

Hecht, S.S., Chung, F.L., Ritchie, J.P., Akerkar, S.A., Borukhova, A., Skowronski, L., Carmella, S.G., Effects of watercress consumption on metabolism of a tobacco-specific lung carcinogen in smokers, *Cancer Epidemiol. Biomarkers Prev.* 4: 877-884 (1995).

Hoffmann, D., Djordjevic, M.V., Rivenson, A., Zhang, E., Desai, D., Amin, S., A study of tobacco carcinogenesis. II. Relative potencies of tobacco-specific N-nitrosamines as inducers of lung tumours in A/J mice, *Cancer Lett.* 71: 25-30 (1993).

Hoffmann, D., Brunnemann, K.D., Prokopczyk, B., Djordjevic, M.V., Tobacco-specific N-nitrosamines and areca-derived N-nitrosamines: Chemistry, biochemistry, carcinogenicity, and relevance to humans, *J. Toxicol. Environ. Health* 41: 1-52 (1994).

Hong, J.Y., Smith, T.J., Lee, M.J., Li, W., Ma, B.L., Ning, S.M., Brady, J.F., Thomas, P.E., Yang, C.S., Metabolism of carcinogenic nitrosamines by rat nasal mucosa and the effects of dially sulfide, *Cancer Res.* 51: 1509-1514 (1991).

Hong, J.Y., Wang, Z.Y., Smith, T.J., Zhou, S., Shi, S., Pan, J., Yang, C.S., Inhibitory effects of dially sulfide on the metabolism and tumorigenicity of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *Carcinogenesis* 13: 901-904 (1992).

PM3003531153

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

International Agency for Research on Cancer, IARC Monographs on the Carcinogenic Risk of Chemicals to Humans, Vol. 37, *Tobacco Habits Other than Smoking; Betel-Quid and Areca-Nut Chewing; and Some Related Nitrosamines*. International Agency for Reesearch on Cancer, Lyon, (1985).

Jorquera, R., Castonguay, A., Schuller, H.M., Effect of tobacco smoke condensate on the metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by adult and fetal hamster microsomes, *Drug. Met. Dis.* 21: 318-324 (1993).

Kim, P.M., Wells, P.G., Genoprotection by UDP-glucuronosyltransferases in peroxidase-dependent, reactive-oxygen species-mediated micronucleus initiation by the carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and benzo[a]pyrene, *Cancer Res.* 56: 1526-1532 (1996).

Klus, H., Begutter, H., Scherer, G., Tricker, A.R., Adlkofer, F., Tobacco-specific and volatile N-nitrosamines in environmental tobacco smoke of offices, *Indoor Environ.* 1: 348-350 (1992).

Lacroix, D., Desrochers, M., Castonguay, A., Anderson, A., Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in human kidney epithelial cells transfected with rat CYP2B1 cDNA, *Carcinogenesis* 14: 1639-1642 (1993).

Lin, J.M., Desai, D.H., Morse, M.A., Amin, S., Hecht, S.S., Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone pulmonary metabolism and tumorigenicity in mice by analogues of the investigational chemotherapeutic drug 4-ipomeanol, *Chem. Res. Toxicol.* 5: 674-679 (1992).

Liu, L., Alaoui-Jamali, M.A., El Alami, N., Castonguay, A., Metabolism and DNA single strand breaks induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and its analogues in primary culture of rat hepatocytes, *Cancer Res.* 50: 1810-1816 (1990).

Liu, L., Castonguay, A., Gerson, S.L., Lack of correlation between DNA methylation and hepatogenesis in rats and hamsters treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *Carcinogenesis* 13: 2137-2140 (1992).

Liu, Y., Sundqvist, K., Belinksy, S.A., Castonguay, A., Tjalve, H., Grafström, R.C., Metabolism and macromolecular interaction of the tobacco-speciific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in cultured explants and epithelial cells of human buccal mucosa, *Carcinogenesis* 14: 2382-2388 (1993).

Maser, E., Richter, E., Friebershauser, J., The identification of 11 β-hydroxysteroid dehydrogenase as carbonyl reductase of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *Eur. J. Biochem.* 238: 484-489 (1996).

PM3003531154

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Merger, M., Meger-Kossien, I., Scherer, G., Metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in urine of smokers and nonsmokers, *Proc. Am. Assoc. Cancer Res.*, 40, Abs. 2275 (1998).

Miller, C.H., Zhang, Z., Hamilton, S.M., Teel, R.W., Effects of capsaicin on liver microsomal metabolism of the tobacco-specific nitrosamine NNK, *Cancer Lett.* 75: 45-52 (1993).

Miller, C.H., Hamilton, S.M., Teel, R.W., Effects of compounds of plant origin on the mutagenicity and metabolism of the tobacco-specific nitrosamine NNK, *Phytotherapy Res.* 8: 342-347 (1994).

Miller, C.H., Castonguay, A., Teel, R.W., Modulation of the mutagenicity and metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by phenolic compounds, *Mutation Res.* 368: 221-233 (1996).

Morse, M.A., Ekling, K.I., Toussaint, M., Amin, S.G., Chung, F.L., Characterization of a glucuronide metabolie of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and its dose-dependent excretion in the urine of mice and rats, *Carcinogenesis* 11: 1819-1823 (1990).

Morse, M.A., Kresty, L.A., Toburen, A.L., Inhibition of metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by dietary benzaldehyde, *Cancer Lett.* 97: 255-261 (1995).

Murphy, S.E., Heiblum, R., Effect of nicotine and tobacco-specific nitrosamines on the metabolism of N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by rat oral tissue, *Carcinogenesis* 11: 1663-1665 (1990).

Murphy, S.E., Coletta, K.A., Two types of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone hemoglobin adducts, from metabolites which migrate into or are formed in red blood cells, *Cancer Res.* 53: 777-783 (1993).

Murphy, S.E., Palomino, A., Hecht, S.S., Hoffmann, D., Dose-response study of DNA and hemoglobin adduct formation by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in F344 rats, *Cancer Res.* 50: 5446-5452 (1990a).

Murphy, S.E., Heiblum, R., Trushin, N., Comparative metabolism of N'-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by cultured rat oral tissue and esophagus, *Cancer Res.* 50: 4685-4691 (1990b).

Murphy, S.E., Heiblum, R., Kling, P.G., Bowman, D., Davis, W.J., Stoner, G., Effect of phenethyl isothiocyanate on the metabolism of tobacco-specific nitrosamines by cultured rat oral tissue, *Carcinogenesis* 12: 957-961 (1991).

PM3003531155

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Murphy, S.E., Spina, D.A., Nunes, M.G., Puilo, D.A., Glucuronidation of 4-((hydroxymethyl) nitrosamino)-1-(3-pyridyl)-1-butanone, a metabolically activated form of 4-(methylnitrosamino)-butanone, by pherobarbital-treated rats, *Chem. Res. Toxicol.* 8: 772-779 (1995).

Patten, C.J., Smith, T.J., Murphy, S.E., Wang, M.H., Lee., J., Tynes, R.E., Koch, P., Yang, C.S., Kinetic analysis of the activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by heterologously expressed human P450 enzymes and the effects of P450-specific chemical inhibitors on this activation in human liver microsomes, *Arch. Biochem. Biophys.* 333: 127-138 (1996).

Pepin, P., Rossignol, G., Castonguay, A., Inhibition of NNK-induced lung tumorigenesis in A/J mice by ellagic acid and butylated hydroxyanisole, *Cancer J.* 3: 266-272 (1990).

Pepin, P., Bouchard L., Nicole, P., Castonguay, A., Effects of sulindac and oltipraz on the tumorigenicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung, *Carcinogenesis* 13: 341-348 (1992).

Peterson, L.A., Carmella, S.G., Hecht, S.S., Investigations of metabolic precursors to hemoglobin and DNA adducts of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *Carcinogenesis* 11: 1329-1333 (1990).

Peterson, L.A., Hecht, S.S, O^6-methylguanine is a critical determinant of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone tumorigeneiss in A/J mouse lung, *Cancer Res.* 51: 5557-5564 (1991).

Peterson, L.A., Mathew, R., Hecht, S.S., Quantification of microsomal α-hydroxylation of the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, *Cancer Res.* 51: 5495-5500 (1991).

Preussmann, R., Steward B.W., N-nitroso carcinogens. In: Searle, C.E. (Ed.), *Chemical Carcinogens*, 2nd Edition. ACS Monograph Series No. 182, American Chemical Society, Washington, D.C., pp. 643-823 (1984).

Richter, E., Tricker, A.R., Nicotine inhibits the metabolic activation of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in rats, *Carcinogenesis* 15: 1061-1064 (1994).

Richter E., Branner, B., Kutzer, C., Donhärl, A.M.E., Scherer, G., Tricker, A.R., Heller, W.D., Comparison of biomarkers for exposure to environmental tobacco smoke: Cotinine and hemoglobin adducts from aromatic amines and tobacco-specific nitrosamines in pregnant smoking and nonsmoking women. In: Maroni M. (Ed.), *Healthy Buildings '95*, Vol. 2, pp. 611-616 (1995).

Schulze, J., Schläger, W. Wünch, R., Richter, E., Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by hamster, mouse and rat intestine: Relevance of species differences, *Carcinogenesis* 17: 1093-1099 (1996).

PM3003531156

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Smith, T.J., Guo, Z., Thomas, P.E., Chung, F.L., Morse, M.A., Eklind, K., Yang, C.S., Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in mouse lung microsomes and its inhibition by isothiocyanates, *Cancer Res.* 50: 6817-6822 (1990).

Smith, T.J., Guo, Z., Gonzalez, F.J., Guengerich, F.P., Stoner, G.D., Yang, C.S., Metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in human lung and liver microsomes and cytochrome P-450 expressed in hepatoma cells, *Cancer Res.* 52: 1757-1763 (1992).

Smith, T.J., Guo, Z., Li, C., Ning, S.M., Thomas, P.E., Yang, C.S., Mechanisms of inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation in mouse by dietary phenethyl isothiocyanates, *Cancer Res.* 53: 3276-3282 (1993).

Smith, T.J., Stoner, G.D., Yang, C.S., Activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in human lung microsomes by cytochrome P450, lipoxygenase, and hydroperoxides, *Cancer Res.* 55: 5566-5573 (1995).

Smith, T.J., Liao, A.M., Liu, Y., Jones, A.B., Anderson, L.M., Yang, C.S., Enzymes involved in the bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in patas monkey lung and liver microsomes, *Carcinogenesis* 18: 1577-1584 (1997).

Spratt, T.E., Trushin, N., Lin, D., Hecht, S.S., Analysis of N^2-(pryidyloxobutyl)deoxyguanosine adducts in DNA of tissues of exposed to tritium-labeled 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and N'-nitrosonornicotine, *Chem. Res. Toxicol.* 2: 169-173 (1989).

Staretz, M.E., Hecht, S.S., Effects of phenethyl isothiocyanate on the tissue distribution of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and metabolites in F344 rats, *Cancer Res.* 55: 5580-5588 (1995).

Staretz, M.E., Murphy, S.E., Patten, C.J., Nunes, M.G., Koehl, W., Amin, S., Koenig, L.A., Guengerich, F.P., Hecht, S.S., Comparative metabolism of the tobacco-related carcinogens benzo[a]pyrene, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and N'-nitrosonornicotine in human hepatic microsomes, *Drug Metab. Dis.* 25: 154-162 (1997a).

Staretz, M.E., Foiles, P.G., Miglietta, L.M., Hecht, S.S., Evidence for an important role of DNA pyridyloxobutylation in rat lung carcinogenesis by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone: Effects of dose and phenethyl isothiocyanate, *Cancer Res.* 57: 259-266 (1997b).

Tjälve, H., Castonguay, A., Distribution and metabolism in Syrian golden hamsters of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobaccl-specific carcinogen. In: Hayes, A.W., Schnell, F.C., Miva, T.S. (Eds.), *Developments in the Science and Practice of Toxicology*, Elsevier Science Publishers, Amsterdam, pp. 423-427 (1983).

PM3003531157

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Tricker, A.R., N-nitroso compounds and man: Sources of exposure, endogenous formation and occurrence in body fluids, *Eur. J. Cancer Prev.* 6: 226-268 (1997).

Tricker, A.R., Ditrich, C., Preussmann, R., N-Nitroso compounds in cigarette tobacco and their occurrence in mainstream cigarette smoke, *Carcinogenesis* 12: 257-261 (1991).

Tricker, A.R., Klus, H., Begutter, H., Ruppert, T., Scherer, G., Adlkofer, F., Tobacco-specific and volatile N-nitrosamines in indoor air of smoker and nonsmoker homes. In: Weber, L. (Ed.), *Indoor Air Pollution*, Franz Siegel Buch GmbH, Ulm, pp. 310-316 (1994).

Vickers, A.E.M., Use of organ slices to evaluate the biotransformation and drug-induced side-effects of pharmaceuticals, *Cell Biol. Toxicol.* 10: 407-414 (1994).

Yoo, J.S.H., Smith, T.J., Ning, S.M., Lee, M.J., Thomas, P.E., Yang, C.S., Modulation of the levels of cytochromes P450 in rat liver and lung by dietary lipid, *Biochem. Pharmacol.* 43: 2535-2542 (1992).

Zhang, Z., Huynh, H., Teel, R.W., Effects of orally administered capsaicin, the principal component of capsicum fruits, on the in vitro metabolism of the tobacco-specific nitrosamine NNK in hamster lung and liver microsomes, *Anticancer Res.* 17: 1093-1098 (1997).

PM3003531158

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Appendix I. *In vitro* metabolism of NNK in tissues, cultured cells and microsomes from laboratory animals and man

Species	Tissue[1]	Conditions		Percentage formation of major metabolites[2]								Percentage α-hydroxylation[3]	Reference
		NNK (μM)	Time (h)	NNAL	NNK-N-oxide	NNAL-N oxide	NNAL-Gluc	Keto alcohol	Keto acid[4]	Hydroxy acid	Diol		
A/J mouse	Lung (t)	4.7	4.0	1.2	49.8	0.8	--	27.1	19.5	0.8	0.8	47.4	Pepin et al., 1992
A/J mouse	Lung (t)	4.7	0.8	0.6	53.7	1.3	--	27.6	13.4	1.3	2.1	42.3	Pepin et al., 1992
A/J mouse	Lung (t)	2.36	2.0	1.6	53.9	0.6	--	29.7	12.4	0.4	1.4	42.5	Bouchard & Castonguay, 1993
A/J mouse	Lung (t)	0.24	24	68.4	7.5	3.7	--	3.1	10.6	1.5	5.2	15.2	Castonguay et al., 1983a
A/J mouse	Lung (m)	200.0	0.5	76.3	10.4	--	--	--	2.5	--	10.8	2.5	Peterson et al., 1991
A/J mouse	Lung (m)	10.0	0.5	12.5	43.0	2.0	--	24.7	(17.1)	--	--	41.8	Smith et al., 1993
A/J mouse	Lung (m)	10.0	0.5	16.3	31.8	--	--	49.7	2.2	--	--	51.9	Smith et al., 1990
A/J mouse	Lung (m)	10.0	0.25	13.7	39.1	--	--	32.7	14.5	--	--	47.2	Lin et al., 1992
A/J mouse	Lung (m)	10.0	0.25	17.8	42.2	--	--	26.2	(13.8)	--	--	40.0	Desai et al., 1995
A/J mouse	Lung (m)	10.0	0.16	5.6	45.6	--	--	29.6	(19.2)	--	--	48.8	Hong et al., 1992
A/J mouse	Lung (m)	--	0.6	29.4	21.7	--	--	48.7	--	--	--	48.7	Morse et al., 1995
A/J mouse	Liver (m)	10.0	0.5	17.7	13.1	--	--	28.9	(40.3)	--	--	69.2	Hong et al., 1992
A/J mouse	Liver (m)	10.0	0.25	53.3	--	--	--	16.3	30.4	--	--	46.7	Lin et al., 1992
A/J mouse	Liver (m)	10.0	0.25	24.0	--	--	--	23.2	(52.8)	--	--	47.2	Desai et al., 1995
A/J mouse	Liver (m)	10.0	0.16	26.6	4.7	3.1	--	24.4	(41.1)	--	--	65.5	Smith et al., 1993
A/J mouse	Liver (m)	--	0.25	22.9	--	--	--	27.8	49.3	--	--	77.1	Morse et al., 1995
A/J mouse	Intestine (t)	14.0	0.75	87.8	2.9	0.8	--	2.7	5.4	0.3	0.1	8.4	Pepin et al., 1990
A/J mouse	Stomach (t)	14.0	2.0	67.2	8.0	0.3	--	5.7	18.2	0.3	0.3	24.2	Pepin et al., 1990
NMRI (f)	Intestine (t)	1.0	2.0	4.8	28.9	8.1	--	2.4	44.9	10.8	--	58.1	Schulze et al., 1996
NMRI (m)	Intestine (t)	1.0	2.0	7.1	29.5	4.7	--	4.2	50.6	3.9	--	58.7	Schulze et al., 1996
F344 rat	Lung (m)	1.0	0.5	22.9	38.3	--	--	34.4	4.4	--	--	38.8	El-Bayoumy et al., 1996
F344 rat	Liver (h)	5.0	2.0	41.7	6.1	0.5	--	6.4	35.4	9.0	0.9	50.8	Murphy & Coletta, 1993
F344 rat	Liver (h)	1.0	18.0	11.4	2.8	7.9	12.5	3.7	44.7	17.0	--	65.4	Murphy et al., 1995
F344 rat	Liver (m)	1.33	0.5	99.6	0.2	0.004	--	--	0.2	0.01	0.04	0.21	Hamilton & Teel, 1994
F344 rat	Liver (m)	1.0	0.5	98.9	0.1	--	--	0.7	0.3	--	--	1.0	El-Bayoumy et al., 1996
F344 rat	Esoph. (t)	40.0	24.0	91.3	4.5	--	--	0.7	3.5	--	--	4.2	Murphy et al., 1990b
F344 rat	Nasal (t)	23.8	3.0	5.4	--	--	--	21.3	64.0	5.3	4.0	70.6	Brittebo et al., 1983
F344 rat	Nasal (t)	23.8	24.0	4.9	--	--	--	5.6	69.9	11.2	8.4	86.7	Brittebo et al., 1983
F344 rat	Oral (t)	1.0	24.0	19.5	51.0	2.0	--	16.3	11.2	--	--	27.5	Murphy et al., 1990b
F344 rat	Oral (t)	1.0	24.0	26.2	36.3	8.8	--	6.9	20.7	0.6	0.5	28.2	Murphy et al., 1990b

PM3003531159

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

F344 rat	Oral (t)	1.0	24.0	55.0	25.2	--	--	9.0	8.3	0.4	2.2	17.7	Murphy et al., 1991
F344 rat	Oral (t)	1.0	24.0	49.9	21.5	5.5	--	9.1	11.5	0.7	1.8	21.3	Murphy & Heiblum, 1991
F344 rat	Oral (t)	1.0	4.0	35.8	3.9	30.6	--	14.6	13.6	1.5	--	29.7	Murphy et al., 1990b
F344 rat	Oral (t)	1.0	4.0	50.8	20.5	5.1	--	8.7	14.3	0.6	--	23.6	Murphy et al., 1990b
F344 rat	Oral (t)	11.0	4.0	81.9	5.5	--	--	3.1	9.5	--	--	12.6	Murphy et al., 1990b
SD rat	Lung (m)	10.0	0.5	28.6	24.4	0.5	--	46.0	0.5	--	--	46.5	Yoo et al., 1992
SD rat	Lung (m)	0.47	0.33	25.7	46.3	--	--	15.5	12.5	--	--	28.0	Ardries et al., 1996
SD rat	Liver (m)	10.0	0.5	93.6	1.3	0.2	--	3.9	1.0	--	--	4.9	Yoo et al., 1992
SD rat	Nasal (m)	10.0	0.5	--	1.0	1.0	--	58.5	36.6	0.9	2.0	96.0	Hong et al., 1991
SD rat (f)	Intestine (t)	1.0	2.0	6.6	29.4	6.9	--	10.3	4.2	2.7	--	17.2	Schulze et al., 1996
SD rat (f)	Intestine (t)	1.0	2.0	78.2	11.2	1.1	--	3.2	6.0	0.4	--	9.6	Schulze et al., 1996
SG Hamster	Lung (t)	4.2	3.0	20.4	32.3	1.8	--	17.9	25.5	0.3	1.8	43.7	Charest et al., 1989
SG Hamster	Lung (t)	0.66	1.0	37.4	27.4	4.7	--	12.0	16.5	0.5	1.5	29.0	Tjälve & Castonguay, 1983
SG Hamster	Lung (t)	0.24	3.0	22.1	33.2	5.5	--	17.7	20.7	0.7	--	39.1	Charest et al., 1989
SG Hamster	Lung (m)	10.0	0.5	50.5	19.6	1.9	--	24.3	3.7	--	--	28.5	Jorquera et al., 1993
SG Hamster	Lung (m)	--	0.5	13.0	16.5	7.2	--	40.6	9.8[5]	--	12.9	50.4	Zhang et al., 1997
SG Hamster	Liver (t)	0.66	1.0	86.0	1.2	3.6	--	--	2.9	3.1	3.2	6.0	Tjälve & Castonguay, 1983
SG Hamster	Liver (m)	10.0	0.5	60.2	9.0	0.7	--	24.1	6.0	0.03	--	30.1	Jorquera et al., 1993
SG Hamster	Liver (m)	8.0	0.5	44.5	8.7	3.2	--	35.5	4.0[5]	--	4.1	59.5	Miller et al., 1994
SG Hamster	Liver (m)	5.1	0.5	69.9	12.7	0.9	--	6.8	1.4[5]	--	1.3	8.2	Miller et al., 1993
SG Hamster	Liver (m)	5.1	0.5	67.7	8.3	4.6	--	12.0	6.1[5]	0.1	1.2	18.2	Miller et al., 1996
SG Hamster	Liver (m)	1.33	0.5	55.4	19.6	2.9	--	17.5	0.8[5]	--	3.9	18.3	Hamilton & Teel, 1994
SG Hamster	Liver (m)	1.0	1.0	62.7	2.4	--	--	30.6	3.3	0.1	0.9	34.0	Castonguay & Rossignol, 1992
SG Hamster	Liver (m)	--	0.5	31.1	6.1	6.4	--	24.9	22.1[5]	0.4	9.0	47.4	Zhang et al., 1997
SG Hamster	Nasal (t)	0.66	1.0	21.0	1.8	2.0	--	13.2	51.3	2.2	8.5	66.7	Charest et al., 1989
SG Hamster	Trachea (t)	0.66	1.0	31.1	11.5	--	--	18.2	38.0	--	1.2	56.2	Charest et al., 1989
SG Hamster	Intestine (t)	1.0	2.0	11.0	25.8	31.1	--	5.8	14.6	11.7	--	32.1	Schulze et al., 1996
Man	Lung (t)	238.0	24.0	96.4	2.7	--	--	--	--	0.9	--	0.9	Castonguay et al., 1983b
Man	Lung (m)	10.0	1.0	96.4	1.2	<0.2	--	<0.2	(1.4)	0.7	--	2.3	Smith et al., 1992
Man	Lung (m)	10.0	0.17	96.1	--	--	--	-------(3.0)------		0.9	--	3.9	Smith et al., 1995
Man	Liver (m)	3.0	0.17	83.5	1.0	--	--	7.5	7.0[5]	--	1.0	14.5	Staretz et al., 1997a
Man	Liver (m)	10.0	1.0	92.4	1.2	0.7	--	3.3	(1.5)	0.9	--	5.7	Smith et al., 1992
Man	Bladder (t)	238.0	24.0	99.9	--	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	Bronchus (t)	238.0	24.0	95.4	4.5	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	Oral (t)	238.0	24.0	99.2	0.1	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	Oral (t)	6.0	24.0	94.8	0.7	1.5	--	0.2	0.5	1.8	0.6	2.5	Liu et al., 1993
Man	Oral (t)	100.0	24.0	99.5	0.2	0.1	--	0.1	0.3	0.1	0.1	0.5	Liu et al., 1993
Man	Oral ©	6.0	24.0	94.7	1.2	0.4	--	0.8	2.1	0.4	0.5	3.3	Liu et al., 1993

0002296.01

PM3003531160

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001

Man	Oral ©	100.0	24.0	96.2	0.6	0.2	--	0.3	2.0	0.2	0.1	2.5	Liu et al., 1993
Man	Esophagus (t)	238.0	24.0	98.2	1.7	--	--	--	--	0.1	--	0.1	Castonguay et al., 1983b
Man	Kidney ©	2.3	50.0	99.8	0.03	--	--	--	0.05	0.06	0.03	0.11	Lacroix et al., 1992
Man	Trachea (t)	238.0	24.0	94.2	0.1	--	--	--	--	5.7	--	5.7	Castonguay et al., 1983b

1. Abbreviation in brackets: (t), tissue; ©, cultured cell; (h), hepatocyte; (m), microsome; --, not detected.

2. Calculated on the basis of total metabolites detected.

3. % α-hydroxylation calculated from the sum of keto alcohol, keto acid (keto aldehyde) and hydroxy acid; metabolic pathways known to produce reactive intermediates which bind to DNA.

4. Keto aldehyde measured instead of keto acid, value for keto aldehyde presented in brackets.

5. Joint determination of keto aldehyde and keto acid.

PM3003531161

Source: https://www.industrydocuments.ucsf.edu/docs/njnj0001